

APPENDIX TO THE AMENDMENT

Please amend the specification as follows:

Replace the second full paragraph on page 43, continuing onto page 44, with the following new paragraph:

Preferred antisense polynucleotides according to the present invention are complementary to a sequence of the mRNAs of P95/nucleolin, P40/PHAPII or P30/PHAPI that contains the translation initiation codon ATG. As an illustrative embodiment of such preferred antisense polynucleotides are 30 mer polynucleotides that are complementary to the following cDNA sequences:

- a) P95/nucleolin: 5'-CGCCGCCATC ATGGTGAAGC TCGCGAAGGT-3' (SEQ ID NO: 1), which corresponds to the cDNA sequence beginning at nucleotide in position 1161 and ending at nucleotide in position 1190 of the nucleic sequence shown in Figure 49, Section II.
- b) P30/PHAPI: 5'-GAGAGCGCGA GAGATGGAGA TGGGCAGACG-3' (SEQ ID NO: 2), which corresponds to the cDNA sequence beginning at nucleotide in position 91 and ending at nucleotide in position 120 of the nucleic sequence shown in Figure 49, Section III.
- c) P40/PHAPII: 5'-GCAGCACCAT GTCGGCGCCG GCGGCCAAAG-3' (SEQ ID NO: 3), which corresponds to the cDNA sequence beginning at nucleotide in position 11 and ending at nucleotide in position 40 of the nucleic sequence shown in Figure 49, Section IV.

Page 47, please replace the fourth full paragraph, continuing onto page 48, with the following new paragraph:

As an illustrative example, a pair of primers used to quantitate P95/nucleolin, P40/PHAPII or P30/PHAPI reverse-transcribed mRNA is the following:

a) P95/nucleolin

Sense primer: 5'-CTTCGGGTGTACGTGCTCCGGG-3' (SEQ ID NO: 4), which is complementary to a sequence beginning at the nucleotide in position nt 1070 and ending at the nucleotide in position 1091 of the nucleic sequence reported in Figure 49, Section II.

Antisense primer: 5'-CCTGAGTGACTTTGTAAGGGAG-3' (SEQ ID NO: 5), which corresponds to a sequence beginning at the nucleotide in position nt 7069 and ending at the nucleotide in position nt 7090 of the nucleic sequence reported in Figure 49, Section II.

Specific probe: a polynucleotide having the nucleic sequence of the amplicon itself.

b) P30/PHAPI

Sense primer: 5'-CCGCCGGCGCGGCAGCCTCTG-3' (SEQ ID NO: 6), which is complementary to a sequence of the nucleic sequence reported in Figure 49, Section III.

Antisense primer: 5'-GTCATCATCTTCTCCCTCATC-3' (SEQ ID NO: 7), which corresponds to a nucleic sequence of the nucleic sequence reported in Figure 49, Section III.

Specific probe: a polynucleotide having the nucleic sequence of the amplicon itself.

c) P40/PHAPII

Sense primer: 5'-CGACCGCGGAGCAGCACCATC-3' (SEQ ID NO: 8), which is complementary to a sequence of the nucleic sequence reported in Figure 49, Section IV.

Antisense primer: 5'-GGAAGGTTGGAATCCATCAG-3' (SEQ ID NO: 9), which corresponds to a sequence of the nucleic sequence reported in Figure 49, Section IV.

Specific probe: a polynucleotide having the nucleic sequence of the amplicon itself.

Page 96, please replace the first paragraph, continuing onto page 97, with the following paragraph:

The V3 loop sequence corresponded to that from the HIV-1 Lai isolate (Myers et al., 1994). It contained 40 amino acids:

NCTRPNNNTRKSIRIQRGPGRAFVTIGKIGNMRQAHCNIS (SEQ ID NO: 10). The other V3 loop sequence corresponded to that from the HIV-1 Ba-L which 39 amino acids sequence is: NCTRPNNNTRKSIHIGPGRAFYTTEIIGDIRQAHCNLS (SEQ ID NO:

11). The biotin-labeled V3 loop peptide was synthesized using classical Fmoc chemistry. Assembly of the protected peptide chain was carried out on a 25 mmol scale; the starting Fmoc Ser (tBu) wang resin is commercially available. The protecting groups for the side chains were tBu (Ser, Thr), Trt (Asn, Glu, His, Cys), Pmc (Arg), Boc (Lys). Assembly of the amino acids was realized according to a procedure described previously, using a multichannel peptide synthesizer (Neimark and Briand, 1993). Biotin was coupled to the peptide according to the procedure used to couple amino acids (thus

the biotin was at the NH₂-terminal of the peptide) and after the last step of deprotection. The biotinyl-V3 loop peptide-resin was then washed 3 times with dichloromethane, dried using ether, deprotected and cleaved from the resin using 6 ml of King's reagent (King et al., 1990). The total cleavage time was 2 h 30 min. The cleaved peptide was filtered before precipitation using cold (0°C) ether. After centrifugation, the pellet was washed twice (10 min each time) with ether. After the last centrifugation, the pellet was washed twice (10 min each time) with ether. After the last centrifugation, the pellet was solubilized in 15 ml of 10% acetic acid (v/v), and then in 1000 ml of water. The pH of the solution was raised to 9 using 1N NaOH. Taking advantage of the cysteine residues at the NH₂- and COOH-termini of the peptide, the loop structure was generated by air oxidation for 3 days under vigorous stirring. Finally, the pH was adjusted to 4 and the cyclized peptide was concentrated on a C₁₈ column eluted with 60% acetonitrile in water and 0.1% trifluoroacetic acid. After lyophilization, the crude cyclized peptide (100 mg) was purified by a semi-preparative HPLC system (ABI Perkin Elmer) using a prep-10 Brownlee column (1 x 10 cm; particle size of 20 mm) and a gradient of acetonitrile 0% to 80% in 0.1 trifluoroacetic acid. The final product (15 mg) was 91% pure with a mass M+H⁺ of 4706.72; the expected mass being 4707.

Please replace the second full paragraph on page 116, continuing onto page 117, with the following new paragraph:

PHAP I and PHAP II had been isolated as putative HLA Class II associated proteins because of their affinity to bind specifically to a synthetic peptide corresponding

to the cytoplasmic COOH-terminal domain of MHC class II DR2a but not to DR2b chain (Vaesen et al., 1994). The predominant structural feature of both PHAP I and PHAP II is a long stretch of acidic amino acids composed of aspartate and glutamate residues at their C-Terminal ends (Vaesen et al., 1994). Microsequencing several peptides from P30 and P40 revealed their identity as PHAP I and PHAPII, respectively (Table 3). In addition to the amino acid sequence homology, the migration profile of P30 and P40 observed in PAGE/SDS, corresponded well with the reported profile of PHAP I and PHAP II (Vaesen et al., 1994). It should be noted that the 14 amino acid sequence of the peak 29 from P30, (K)KLELSENRIFGGL (SEQ ID NO: 12) (Table 3) is homologous to the amino acid fragment KKLELSDNRVSGGL (SEQ ID NO: 13) at position 67 to 80 in PHAP I. The differences between the deduced amino acid sequence of PHAP I and the sequence obtained by microsequencing are D/ to E, V to I, and S to F. Considering the genetic code for these amino acids, a single error in the nucleotide sequence might have accounted for this difference. As the PHAP I cDNA was obtained after PCR amplification using degenerated primers, it is plausible to suggest that some errors might have been generated during the amplification process (Vaesen et al., 1994).

Page 160, please replace Table 3 with the following new Table:

Table 3: Homology of the amino acid sequence of the different peptides from the V3 loop BPs to nucleolin, PHAP II and PHAP I.			
Protein	Peptide Fractions	Amino Acid Sequence	Homology (a-a)
A: p95	Peak 24	(K)QGTEIDGRSISLYYT (SEQ ID NO: 14)	Nucleolin (447-563)
	Peak 30	(K)GYAFIEFASFEDA(K) (SEQ ID NO: 15)	Nucleolin (522-536)
A: p60	Peak 24	(K)GYAFIEFASFEDA(K) (SEQ ID NO: 16)	Nucleolin (522-536)
	Peak 18	(K)ALELTG (SEQ ID NO: 17) (K)QGTEID (SEQ ID NO: 18)	Nucleolin (361-367) Nucleolin (447-454)
	Peak 19	(K)VTLDWAKP(K) (SEQ ID NO: 19)	Nucleolin (635-644)
A: p40	Peak 24	(K)EQQEAIEHIDEVQNE (SEQ ID NO: 31)	PHAP II (26-41)
A: p30	Peak 27	(K)KLELSE (SEQ ID NO: 20)	PHAP I (67-73)
	Peak 29	(K)KLELSENRIFGGL (SEQ ID NO: 12)	PHAP I (67-80)
	Peak 33	(K)SLDLFNXEVTNLNDY (SEQ ID NO: 21)	PHAP I (116-131)
B: p95*	NH ₂ -terminal	VKLAKAGKNQGDPKK (SEQ ID NO: 32)	Nucleolin (1-15)

Page 165, please replace Table 7 with the following new table:

Table 7: Inhibition of gp120 binding to the V3 loop-BPs by monoclonal antibodies against the V3 loop.			
mAb	Isotype	Epitope (in gp120) ^a	Inhibition (IC ₅₀) ^b
AD3	IgG2a	NH ₂ -Terminal (aa 1-204)	No Effect ^c
110C	IgG1, κ	FTD (aa 282-284)	\approx 120 nM
V3-21	IgG1, κ	V3: INCTRPN (SEQ ID NO: 29) (aa 298-304)	\approx 100 nM
N11/20	IgG1, κ	V3: GPGRFVFI (SEQ ID NO: 30) (aa 317-325)	\approx 100 nM
110-4	Not known	V3: (aa 303-323)	\approx 100 nM
110-D	IgG2a, κ	(aa 381-394)	No Effect
b12	IgG1	CD4 binding domain	No Effect
ADP390	IgG2b	CD4 binding domain	No Effect
110-1	Not known	COOH-terminal (aa 489-511)	No Effect

Page 168, please replace Table 10 with the following new table:

Table 7: Inhibition of gp120 binding to the V3-BPs by monoclonal antibodies against the V3 loop.			
mAb	Isotype	Epitope (in gp120) ^a	Inhibition (IC ₅₀) ^b
AD3	IgG2a	NH ₂ -Terminal (aa 1-204)	No Effect ^c
110-C	IgG1, κ	FTD (aa 282-284)	\approx 120 nM
V3-21	IgG1, κ	V3: INCTRPN (SEQ ID NO: 29) (aa 298-304)	\approx 100 nM
N11-20	IgG1, κ	V3: GPGRFVFI (SEQ ID NO: 30) (aa 317-325)	\approx 100 nM
110-4	Not known	V3: (aa 303-323)	\approx 100 nM
110-D	IgG2a, κ	(aa 381-394)	No Effect
b12	IgG1	CD4 binding domain	No Effect
ADP390	IgG2b	CD4 binding domain	No Effect
110-1	Not known	COOH-terminal (aa 489-511)	No Effect